

CHROM. 13,839

## Note

### High-performance paper electrophoresis

## II. Comparison of separations obtained by high-voltage paper electrophoresis and high-performance paper electrophoresis

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(Received April 4th, 1981)

During the last few years special attention has been paid to studies on fast separation processes, and so-called "high-performance" methods have been developed in both electrophoresis and chromatography.

Lederer<sup>1</sup> has recently developed a rapid electrophoretic method that can yield good separations within a few minutes by an extremely simple arrangement which has been called "high-performance paper electrophoresis". So far this method has been used experimentally to separate, for example, mixtures of metal ions. We have now carried out a more extensive study and have compared the results with those previously obtained by high-voltage electrophoresis<sup>2-7</sup>.

### EXPERIMENTAL AND RESULTS

The apparatus used consisted of an LKB power supply (Model 337 IE), a plastic electrophoretic cell without a cooling system and glass plates 10 cm long and 5 cm wide and varying in thickness from 2 to 5 mm. The paper strips (Whatman No. 1 or 3MM) were usually 10 cm long and 5-10 mm wide and were placed between the glass plates. Unless indicated otherwise in the figure legends, the length of the experiments varied from 3-15 min. The electric field applied was 40-80 V/cm (in the figure legends we give the values of the voltage applied, as the distance between the electrodes was always 10 cm).

The separations described below were chosen in order to establish the limits of utilization of the method. All of the separations studied had already been achieved by high-voltage electrophoresis<sup>2-7</sup>.

*Separation of cobalt(III) complexes: separation of  $Co(en)_3^{3+}$ ,  $Co(NH_3)_6^{3+}$ ,  $Co(o-phen)_3^{3+}$  and  $Co(dip)_3^{3+}$*

For the separations we used 0.3 and 0.5 N aqueous solutions of sodium trichloroacetate, sodium sulphate and sodium perchlorate. As shown in Figs. 1-4, good separations of all the compounds could be obtained in trichloroacetate, whereas in sulphate and perchlorate solutions  $Co(dip)_3^{3+}$  and  $Co(o-phen)_3^{3+}$  were not separated.

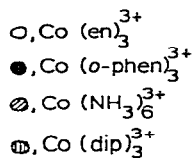
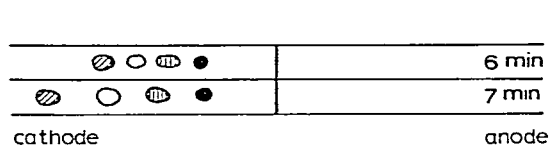


Fig. 1. Electropherograms of cobalt(III) complexes on Whatman 3MM paper at 500 V. Solution of sodium trichloroacetate (0.3 *N*).

Fig. 2. Electropherograms of cobalt(III) complexes on Whatman 3MM paper at 500 V. Solution of sodium trichloroacetate (0.5 *N*). Spots as in Fig. 1.

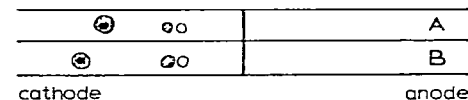
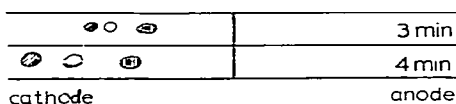


Fig. 3. Electropherograms of cobalt(III) complexes on Whatman 3MM paper at 500 V. Solution of sodium perchlorate (0.3 *N*). Spots as in Fig. 1.

Fig. 4. Electropherograms of cobalt(III) complexes on Whatman 3MM paper at 500 V; time, 6 min. A, Solution of  $\text{Na}_2\text{SO}_4$  (0.3 *N*); B, solution of  $\text{Na}_2\text{SO}_4$  (0.5 *N*).

Figs. 1 and 2 show also that with more concentrated solutions (0.5 *N*) at the same voltage separations could be obtained even in 4 min with a relatively short electrophoretic run.

In all of the experiments we used Whatman 3MM paper because thick paper causes more heating owing to the Joule effect, which favours the separation. As reported previously<sup>2-4</sup>, because of the difference in ion-pair formation, we also observed an inversion of the electrophoretic mobility when we used solutions of sulphate instead of perchlorate and trichloroacetate;  $\text{Co}(\text{III})(\text{en})_3^{3+}$ , for example, was the fastest in perchlorate and trichloroacetate and the slowest in sulphate.

*Separation of cobalt(III) mixed complexes: (a)  $\text{Co}(\text{en})_3^{3+}$ ,  $\text{Co}(\text{en})_2(o\text{-phen})_3^{3+}$ ,  $\text{Co}(\text{en})(o\text{-phen})_2^{3+}$  and  $\text{Co}(o\text{-phen})_3^{3+}$  and (b)  $\text{Co}(\text{en})_3^{3+}$ ,  $\text{Co}(\text{en})_2(\text{dip})_3^{3+}$ ,  $\text{Co}(\text{en})(\text{dip})_2^{3+}$  and  $\text{Co}(\text{dip})_3^{3+}$*

As in the previous instance, we utilized aqueous solutions of trichloroacetate, perchlorate and sulphate. Figs. 5-7 show how it is possible to separate these complexes in all of the solutions examined; the best separations were obtained with 0.5 *N* solutions, but separations could also be obtained with 0.3 *N* solutions; the optimal voltage was 500 V (50 V/cm); the length of the experiments can be reduced to 3 min.

Regarding the series of mixed complexes,  $\text{Co}(\text{en})_3^{3+}$ - $\text{Co}(\text{en})_2(\text{dip})_3^{3+}$ - $\text{Co}(\text{en})(\text{dip})_2^{3+}$ - $\text{Co}(\text{dip})_3^{3+}$ , good separations could be obtained with 0.3 and 0.5 *N* solutions of sodium perchlorate (under different conditions of voltage and time) and with 0.3 and 0.5 *N* solutions of sodium perchlorate (Figs. 8 and 9). In contrast to the results obtained by high-voltage electrophoresis<sup>4</sup>, the four complexes could not be separated in trichloroacetate solutions.

○ ● ρ ●	6 min
○ ● ρ ●	7 min
cathode	anode

○ ● ρ ●	A
○ ● ρ ●	B
cathode	anode

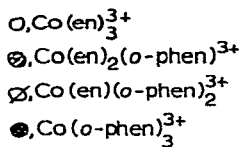


Fig. 5. Separation of mixed complexes of cobalt(III) on Whatman 3MM paper at 500 V. Solution of sodium trichloroacetate (0.5 N).

Fig. 6. Separation of mixed complexes of cobalt(III) on Whatman 3MM paper at 500 V. A, Sodium perchlorate (0.3 N), time = 6 min; B, sodium perchlorate (0.5 N), time = 5 min. Spots as in Fig. 5.

A	
● ρ ● ○	500V
● ρ ● ○	600 V
● ρ ● ○	700V
cathode	anode

B	
● ρ ● ○	3 min
● ρ ● ○	5 min
● ρ ● ○	6 min
cathode	anode

Fig. 7. Electropherograms of mixed complexes of cobalt(III) on Whatman 3MM paper; solution of Na<sub>2</sub>SO<sub>4</sub> (0.5 N). A, Time = 3 min; B, voltage = 500 V. Spots as in Fig. 5.

A	
● ρ ρ ○	600 V
● ρ ρ ○	500 V
cathode	anode
B	
● ρ ρ ○	8 min
● ρ ρ ○	5 min
cathode	anode

A	
○ ρ ρ ●	5 min
○ ρ ρ ●	3 min
cathode	anode
B	
○ ρ ρ ●	5 min
○ ρ ρ ●	3 min
cathode	anode

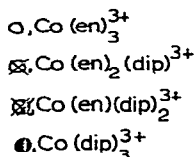


Fig. 8. Electropherograms of mixed complexes of cobalt(III) on Whatman 3MM paper; solution of Na<sub>2</sub>SO<sub>4</sub> (0.3 N). A, Time = 5 min; B, voltage = 500 V.

Fig. 9. Electropherograms of cobalt(III) complexes on Whatman 3MM paper at 500 V. A, Solution of sodium perchlorate (0.3 N); B, solution of sodium perchlorate (0.5 N). Spots as in Fig. 8.

*Separation of some inorganic anions: separation of  $\text{ClO}_3^-$ ,  $\text{BrO}_3^-$  and  $\text{IO}_3^-$*

Fig. 10 shows the separation of chlorate, bromate and iodate ions in 0.1 *N* HCl solutions. At 700 V the time required could be reduced to 60 sec.

● ⊗ ○	180 sec
● ⊗ ○	120 sec
● ○ ○	60 sec
anode	cathode

- ,  $\text{IO}_3^-$   
 ⊗,  $\text{BrO}_3^-$   
 ●,  $\text{ClO}_3^-$

Fig. 10. Separation of some inorganic anions on Whatman No. 1 paper at 700 V. Electrolyte, 0.1 *N* HCl.

*Separation of optical isomers*

We separated optical antipodes of  $\text{Co}(\text{en})_3^{3+}$  with various electrophoretic systems; the results are shown in Figs. 11–14. With solutions of antimonyl tartrate a longer time is necessary (10–15 min). The time required could be considerably reduced (Figs. 13 and 14) if mixtures of aluminium chloride and D(–)- or L(+)-tartrate were used. As we were studying thermolabile substances, we used the lighter Whatman No. 1 paper (which gives a low temperature) or Whatman 3MM paper, and in these instances the glass plates between which the paper strips were placed were much thicker (> 5 mm) in order to absorb the heat generated. With this technique it was impossible to separate the optical isomers of  $\text{Co}(\text{en})_3^{3+}$  with solutions of optically active tartrate, whereas this separation was achieved with high-voltage electrophoresis<sup>6</sup>.

○ ○	(±) $\text{Co}(\text{en})_3^{3+}$ 10 min
○	(±) $\text{Co}(\text{en})_3^{3+}$ 5 min
cathode	anode

Fig. 11. Electropherograms of (±)-cobalt(III)  $(\text{en})_3^{3+}$  on Whatman 3MM paper at 800 V. Electrolyte, antimony potassium (+)-tartrate solution (0.1 *N*).

○ ○	(±) $\text{Co}(\text{en})_3^{3+}$ A
○ ○	(±) $\text{Co}(\text{en})_3^{3+}$ B
cathode	anode

Fig. 12. Electropherograms of (±)-cobalt(III)  $(\text{en})_3^{3+}$  at 500 V for 15 min. Electrolyte, antimony potassium (+)-tartrate solution (0.1 *N*). A, Whatman No. 1 paper; B, Whatman No. 3MM paper.

○ ○	1 (–) $\text{Co}(\text{en})_3^{3+}$
○ ○	2 (±) $\text{Co}(\text{en})_3^{3+}$
○ ○	3 (+) $\text{Co}(\text{en})_3^{3+}$
cathode	anode

Fig. 13. Separation of two enantiomers of  $\text{Co}(\text{en})_3$  on Whatman No. 1 paper at 500 V for 7 min. Electrolyte, mixture of aluminium chloride (0.24 *M*) and disodium D(–)-tartrate (0.36 *M*). The strip paper was 2 cm wide.

○ = ○	(±) $\text{Co}(\text{en})_3^{3+}$ 3 min
○ = ○	(±) $\text{Co}(\text{en})_3^{3+}$ 5 min
○ = ○	(±) $\text{Co}(\text{en})_3^{3+}$ 7 min
cathode	anode

Fig. 14. Separation of two enantiomers of  $\text{Co}(\text{en})_3^{3+}$  on Whatman No. 1 paper at 500 V for 7 min. Electrolyte, mixture of aluminium chloride (0.24 *M*) and disodium D(–)-tartrate (0.36 *M*).

## CONCLUSION

A general comparison of the results obtained by this method with those obtained by high-voltage electrophoresis leads to the following conclusions:

(1) It is possible to obtain separations by high-performance paper electrophoresis whenever the differences in the electrophoretic mobility in high-voltage electrophoresis are 2 cm or more; in this event the time required is reduced from 1 h to about 10 min.

(2) When the mobility differences are less than 1.5–2 cm it is still possible to obtain separations utilizing the increase in the electrophoretic mobility due to the temperature increase. In this instance, the method can only be used with non-thermolabile substances.

(3) Of the different factors that influence the separations, the most important is the variation of temperature due to the Joule effect.

(4) The extreme shortness of the time necessary for the experiments (which generally does not exceed 5 min) makes high-performance paper electrophoresis particularly suitable, for example, for controlling the purity of complexes prepared in the laboratory.

## ACKNOWLEDGEMENT

Thanks are due to Professor M. Lederer for his interest in this work and his helpful suggestions.

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